

Chapter 3

Chickpea

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1 Introduction

Chickpea (*Cicer arietinum* L.) is a unique cultivated species in the genus *Cicer*. It is an annual, self-pollinated crop adapted to post-rainy season either in spring sowing or summer-dominant rainfall regions (Berger and Turner 2007). It has been a food crop since ancient times in the Mediterranean basin from where it was dispersed to the Indian subcontinent becoming a basic constituent of Asian diets.

Nowadays, it is grown all over the five continents in around 50 countries, with 90% of its cultivated area (around 13×10^6 ha) in developing countries. India ranks first in the world in respect of cultivated area (68.5%) followed by Pakistan (8.7%)

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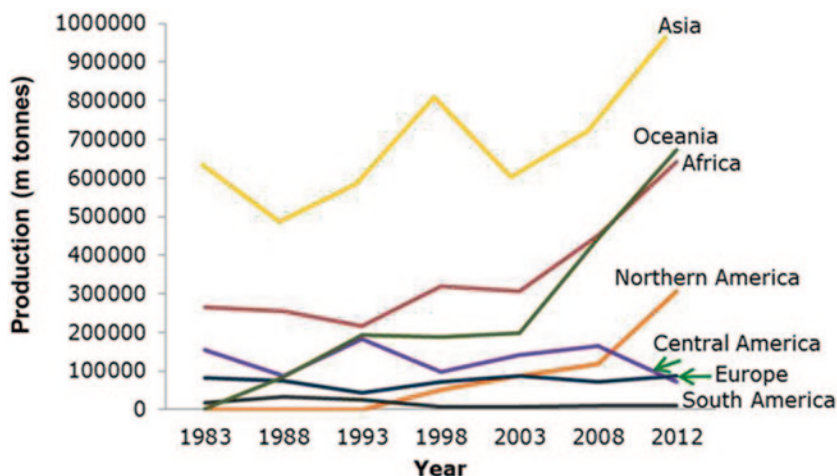


Fig. 3.1 Evolution of world chickpea production

as well as in total production (68%) and followed by Australia (5.9%), that is one of the major exporting countries. This crop is mainly grown under rainfed conditions with a world average yield of 931 kg ha^{-1} in 2012 but reaching a maximum of 6044 kg ha^{-1} in Israel where this crop is grown under irrigated conditions. Chickpea production has increased over the past 30 years from 6.1 to around $13 \times 10^6 \text{ t}$. It has been particularly stunning that the increase of this crop in Africa and Oceania, where the total production was at minimum, doubled (Fig. 3.1). This increase can be explained by (i) the development of new high-yielding varieties tolerant/resistant to main diseases, pests and abiotic stresses and (ii) successful integrated crop management practices. The number of importing countries has increased from 65 in 1991 to 164 in 2011, suggesting the highest global demand for this crop in the past 20 years (Faostat 2014).

Chickpea cropping systems have been recently reviewed by Berrada et al. (2007). In the Mediterranean basin, springtime (February–mid-April) is the traditional sowing date, but with the new winter chickpea varieties, sowing can be advanced to January, whereas in the Indian subcontinent it is sown from mid-September to November after the rainy season. Chickpea has been mainly cultivated in rotation after multiple base crops such as wheat, teff, oat, rice, pearl millet, sorghum, maize or cotton, although intercropping has also been put into practice in subsistence farming areas. Production stability and yields have been limited by various constraints such as susceptibility to major worldwide-distributed diseases, *Ascochyta* blight and *Fusarium* wilt or terminal drought.

This crop, as a legume, improves soil fertility by fixing atmospheric nitrogen, meeting up to 80% of its nitrogen requirement from symbiotic nitrogen fixation (Gaur et al. 2012). Chickpea-specific mesorhizobia are present in soils where chickpea has been traditionally grown but seed inoculation is required in the new chickpea-cultivated lands or in marginal soils. Better N_2 fixation can be achieved

by selecting rhizobial strains of superior N₂-fixing capacity but also depends on chickpea cultivars (Kantar et al. 2007).

Chickpea is a high-quality and cheap source of protein for people in developing countries (particularly in South Asia), who are largely vegetarian. Additionally, it is rich in nutritionally important unsaturated fatty acids and vitamins such as riboflavin, niacin, thiamin, folate and the vitamin A precursor β -carotene. Different studies have provided some evidence to support the potential beneficial effects of chickpea components in lowering the incidence of various cancers, high-density lipoprotein (HDL) cholesterol, type-2 diabetes and heart diseases (Roy et al. 2010; Jukanti et al. 2012).

C. arietinum has a relatively low DNA content estimated to be 738.09 Mb (Varshney et al. 2013) distributed in eight pairs of chromosomes. Genomic tools in chickpea have been progressing very rapidly in the past years. Nowadays, reference genetic maps are available including cross-genome markers from the model species *Medicago truncatula*, which made it possible to assign chickpea linkage groups (LG) to *Medicago* chromosomes. (Millán et al. 2010; Nayak et al. 2010; Gujaria et al. 2011; Thudi et al. 2011). Furthermore, five out of the eight chickpea chromosomes could be isolated by flow-cytometry and specific sequence-tagged microsatellite site (STMS) markers amplified on sorted chromosomes and allowed to assign four linkage groups to particular chromosomes (Vláčilová et al. 2002; Zatloukalová et al. 2011). All this previous information, together with the recent publication of the first draft of the whole-chickpea genome sequence by two different groups (Jain Rajesh et al. 2013; Varshney et al. 2013), provides powerful tools to be used for searching genes involved in agronomic traits. The relatively simple genome of this species together with its importance to world agriculture and available genetic and genomic tools make this crop as a possible “model” for cool-season food-legume genomics.

2 Systematic and Origin

The genus *Cicer* belongs to the family Leguminosae, subfamily Papilionoideae and the monogeneric tribe *Cicereae* Alef., including 35 perennials, 8 annual and 1 unspecified wild species. It is a component of the Galegoid group (or cool season legumes) together with tribes *Viceae*, *Trifoliae* and *Loteae* (Zhu et al. 2005). The tribe *Cicereae* has been subclassified in sections *Cicer* (= *Monocicer*), *Chamaecicer*, *Polycicer* and *Acanthocicer* based on morphological traits and geographical distribution (van der Maesen et al. 2007). The single cultivated species *C. arietinum* belongs to the section *Cicer*.

Based on their crossability with chickpea, the wild *Cicer* annual species have been classified into gene pools reflecting their distance from the cultivated species as proposed by Harlan and de Wet (1971). According to this definition, the primary gene pool consists of *C. arietinum*, the wild annual progenitor, *Cicer reticulatum* Ladz. and the closely related *Cicer echinospermum* P. H. Davis. The secondary gene

pool is composed by *Cicer bijugum* K. H. Rech, *Cicer pinnatifidum* Jaup and Spp. and *Cicer judaicum* Boiss. Sterility is associated with the first-generation hybrids for those species. Interspecific or wide hybridization has been identified as a potential means of increasing the genetic variation and introduction of resistance genes in cultivated species from wild species (Singh et al. 2008).

Chickpea was one of the first domesticated grain legumes together with other crops such as wheat, barley, rye, pea, lentil, flax and vetch in a reduced area of southeast of Turkey (Ladizinsky and Adler 1976; Abbo et al. 2003). The domestication seems to have occurred from the wild ancestor *C. reticulatum* (sin. *C. arietinum* subsp. *reticulatum*) with a monophyletic origin as revealed by the low genetic variation of the cultigen (*C. arietinum* subsp. *arietinum*) (Moreno and Cubero 1978). Only in the early Bronze period (fifth millennium BC) can chickpea be considered as an established crop in the Near East. A few seeds dated ca. 6000 BC have been found in Bulgaria, and two millennia later, in Greece. Thus, it seems that chickpea belonged to the first agricultural complex reaching Europe through the Black Sea (Zohary et al. 2012). Afterward, chickpea was likely taken to India by the Aryan tribes in the second millennium BC who probably brought the crop from Iranian tribes, as suggested by De Candolle on linguistic evidence. It was also spread over the Mediterranean basin and through the Nile River and was expanded to the east of Africa. Chickpea was taken by Spanish colonizers to the New World in 1492. It crossed the Atlantic Ocean in the first Columbus travel of discovery. Chickpea was always a humble crop that almost never appeared on the royal tables, being on the contrary a useful food for both humans and animals and a good companion of man around the globe.

3 Varietal Groups

C. arietinum is divided into two main cultivar groups for breeding purposes ‘desi’ and ‘kabuli’ types. This distinction is made mainly on the basis of a small number of morphological characters, principally the seed shape and colour. White flower, thin seed coat, large seed (200–680 mg) and more of them with a “ram’s head” shape and cream-coloured seeds, smooth seed surface, lack of anthocyanin pigmentation and semi-spreading growth habit are present in the kabuli chickpea. On the other hand, pink flower, thick seed coat, small seed (100–200 mg) angular, dark seeds, anthocyanin pigmentation of stem, rough seed surface and either semi erect or semi-spreading growth habit are characteristics of desi ones (Pundir et al. 1985). This classification overlaps, to a certain extent, with the *macrosperma* and *microsperma* races proposed by Moreno and Cubero (1978) studying quantitative as well as qualitative traits. Additionally, a third type designated as pea-shaped characterized by medium to small seed size and cream-coloured seeds has been proposed (Upadhyaya et al. 2008a).

Desi types, mostly grown in India, Pakistan and East Africa, cover around 85% of chickpea cultivated area. In these countries, seeds are usually dehulled and split

before cooking. Kabuli chickpeas are grown mainly in the Mediterranean basin, the Near East, Central Asia and America where whole seeds are used for human consumption after soaking and boiling. It is believed that the kabuli chickpea was introduced into India through Kabul, Afghanistan (therefore named kabuli), in the mid- to late-seventeenth century (Singh 1987). Kabuli probably evolved from the desi type in the Mediterranean basin and oligogenic traits like flower colour, seed coat thickness and seed size seem to have played an important role in its evolution (Moreno and Cubero 1978; Gil and Cubero 1993).

Significant differences in agronomic traits have been observed between these two groups. Ascochyta blight resistance, cold tolerance and erect growth habit are more frequently found in kabuli types, whereas Fusarium wilt resistance, heat and drought tolerance and early flowering are prevalent in desi types (Singh 1987). They also differ in quality components such as seed coat thickness, crude fibre content, mineral and trace element composition, polyphenolic content and in vitro digestibility (Jambunathan and Singh 1981; Gil et al. 1996). Kabuli types were reported to be nutritionally superior to desi types in terms of cooking time, biological value and sensory properties (Singh et al. 1991) and receive higher market price than desi types. The price premium in kabuli types generally increases as the seed size increases.

The distribution of genetic diversity in kabuli seems to be much narrower than in the desi predominant chickpea type. Both types are also nearly uniform in cytoplasm, indicating no evolution of hybridization barriers (Moreno and Cubero 1978). The desi and kabuli groups tend to have maintained distinct morphological types and may have different gene blocks for important yield components appearing as two separate groups when they are clustered based on molecular marker analysis (Iruela et al. 2002).

These differences have been employed in chickpea-breeding programmes using desi \times kabuli (and vice versa) crosses to obtain new disease-resistant cultivars with higher yields, large seed size and vigour of desi types (Gaur et al. 2007).

4 Genetic Resources and Utilization

The genetic resources provide basic material for selection and improvement through breeding, leading to ensure food security needs of the world's rapidly rising population. They comprise diversity of genetic material contained in traditional varieties, modern cultivars, crop wild relatives and other wild species (Farshadfar and Farshadfar 2008; Upadhyaya et al. 2008b). The collections represent both insurance against genetic erosion and as sources of resistance/tolerance to diseases and pests, climatic and other environmental stresses.

The two major chickpea germplasm collections are maintained at the Consultative Group on International Agricultural Research (CGIAR) centres, the International Crops Research Institute for the Semi-Arid Tropics, <http://www.icrisat.org> (ICRISAT) and the International Center for Agricultural Research in Dryland Areas,

<http://www.icarda.org> (ICARDA) with more than 20,000 and 13,000 accessions, respectively. ICRISAT mainly focusses on desi types while ICARDA maintains mostly kabuli types. Both centres, ICRISAT and ICARDA, maintain wild *Cicer* sp. Other important chickpea collections are conserved by the National Bureau of Plant Genetic Resources, India (NBPGR) with around 14,000 accessions, Centre for Legumes in Mediterranean Agriculture Australia (CLIMA) with approximately 8000 accessions and the United States Department of Agriculture, <http://www.ars-grin.gov> (USDA) with about 6000 accessions (Rubio et al. 2009; Upadhyaya et al. 2011). Other collections have been described by Gaur et al. (2012).

In spite of the large number of germplasm accessions available at the gene banks, there has been a very limited use of these accessions in chickpea breeding; thus, in India, only ten lines contributed to the 35% of genetic base in chickpea (Upadhyaya et al. 2008b). The main reason for the modest utilization of germplasm is the lack of information on a large number of accessions. Thus, core and mini core collections (about 10 and 1% of the total accessions, respectively) have been suggested as an opportunity for the utilization of genetic diversity in crop improvement (Upadhyaya and Ortiz 2001). In chickpea, core and mini core subsets, representative of the entire chickpea collections, have been obtained. Upadhyaya et al. (2001) developed in ICRISAT a chickpea core collection consisting of 1956 accessions, which represented the global chickpea germplasm collection. ICRISAT developed a reference set consisting of 300 accessions representing diversity from the entire spectrum of a composite collection made between ICRISAT and ICARDA (Upadhyaya and Ortiz 2001). Mini core collections have been useful to identify accessions with good agronomic traits and resistance to different diseases (Pande et al. 2006a) as well as in applied breeding for the development of broad-based elite breeding lines/cultivars with superior yield (Upadhyaya et al. 2008b).

The Generation Challenge Program (GCP; www.generationcp.org) is contributing to intensify the molecular characterization of core and mini core collection to identify genetically diverse parents for mapping and utilization in breeding programmes (Gaur et al. 2012).

5 Major Breeding Achievements and Specific Goals in Current Breeding

Breeding efforts have contributed substantially to improve chickpea yield potential but the lack of stable production still continues to be a major concern for the adoption of this crop by farmers. The major constraints limiting chickpea production include various abiotic and biotic stresses, particularly important are fungal diseases (*Ascochyta* blight and *Fusarium* wilt), pests (pod borer) and drought or cold stress. Parasitic plants could also be a big problem in such particular environments such as Mediterranean conditions (Gaur et al. 2007; Chen et al. 2011) (Fig. 3.2).

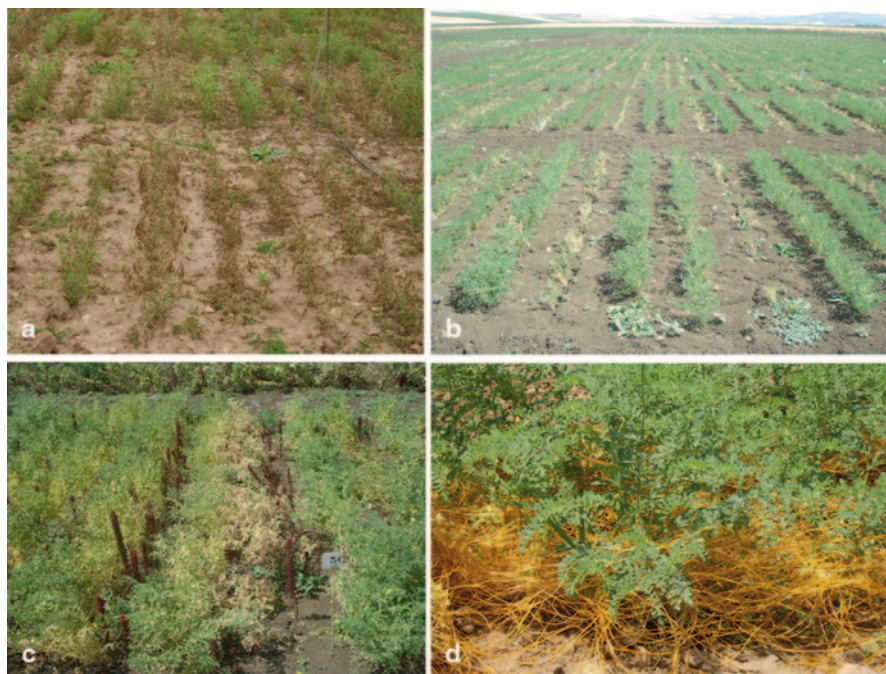


Fig. 3.2 Chickpea screening for resistance/tolerance to different diseases and parasitic plants in specific clinic fields in Tunisia (a) *Ascochyta* blight (b) *Fusarium* wilt (c) Broomrape (*Orobanchae foetida*) (d) Dodder (*Cuscuta* spp.)

5.1 Biotic Stresses

Ascochyta blight, caused by the Ascomycota fungus *Ascochyta rabiei* (Pass.) Labr. (teleomorph *Didymella rabiei* (Kovatsch) Arx.), is one of the most serious diseases of chickpea worldwide, which affects all aerial parts of the plant. It reduces chickpea seed yield significantly reaching 100% of losses when favourable conditions such as cool (5–15 °C) and wet weather occurred (Pande et al. 2005). The use of varieties with improved levels of resistance is considered the most economical solution for long-term disease management. So that, efforts to develop and commercialized blight chickpea resistant cultivars have been made (Gaur et al. 2007; Taran et al. 2013). Currently, the development of blight-resistant lines has made possible the introduction of winter sowing in the Mediterranean region with the prospect of increasing chickpea production that could be doubled (Singh and Reddy 1996). However, reaching high levels of resistance to blight is complex because many genes with minor to moderate effects control this trait and only partial resistance is available (Pande et al. 2005; Taran et al. 2007; Bhardwaj et al. 2010).

The pathogen survives between crop seasons either on infected plant debris or in contaminated seeds. A wide pathogenic variation has been described and those are grouped in two or three pathotype systems (Udupa et al. 1998; Chen et al. 2004,

2011). Breeders try to search for different sources of resistance and pyramid different resistance genes into the same cultivar to improve both the resistance level and the durability of this resistance. Different sources of resistance/tolerance have been employed to achieve this goal, and as a result, a significant number of cultivars with improved Ascochyta blight resistance have been released. This information was summarized in Pande et al. (2010) and Millan et al. (2013). Additional sources of resistance have also been identified among wild *Cicer* species, including *C. judaicum*, *C. pinnatifidum*, *C. echinospermum* and *C. reticulatum* (Pande et al. 2006b).

Fusarium wilt, caused by the vascular pathogen *Fusarium oxysporum* Schlechtend: Fr. f. sp. *ciceris*, is, along with Ascochyta blight, the most important fungal disease in chickpea. Fusarium wilt epidemics can be devastating to individual crops and cause up to 100% loss under favourable conditions. Persistence of the pathogen in soil for many years even in the absence of the host renders its control difficult. Consequently, the use of resistant cultivars is the most economic, effective and ecofriendly method of controlling such pathogen. However, the effectiveness of resistant cultivars is limited by the existence of different races of pathogens. To date, eight physiological races (0, 1A, 1B/C, 2, 3, 4, 5 and 6) have been reported from India, Spain, Tunisia and the USA (Haware and Nene 1982; Jimenez-Gasco et al. 2004). Resistance to wilt in chickpea has been reported to be race-specific and controlled by major resistance genes (Gaur et al. 2007; Sharma and Muehlbauer 2007; Castro et al. 2012a).

The screening of international germplasm has led to the identification of sources of resistance to wilt in both cultivated (desi and kabuli types) and wild chickpea germplasm (Millan et al. 2013). However, the resistance sources in kabuli types are limited compared to the desi types. Haware et al. (1992) evaluated a world collection with 13,500 germplasm accessions for race 1, identifying 160 resistant accessions but only ten of them were kabuli type. The desi type accession WR315 is extensively recognized as possessing resistance to all known wilt races. It has been widely used in inheritance and mapping studies and is considered to be a very interesting source of wilt resistance for chickpea-breeding programmes (Haware 1998). Two kabuli accessions (ICC 14194 and ICC 17109) with complete wilt resistance and extra large seeds, a key quality determinant in the market, were detected by Gaur et al. (2006). Significant progress has been made in Fusarium research and cultivars including resistance to multiple races are now available (Malhotra et al. 2007; Singh et al. 2009).

Botrytis grey mould (BGM) caused by *Botrytis cinerea* Pers. ex. Fr. is the second most important foliar disease of chickpea after Ascochyta. BGM causes complete crop loss in several South Asian countries (Pande et al. 2006c). The limited reports available on genetics of BGM resistance in chickpea suggest that a few major genes control resistance in the host to BGM (Anuradha et al. 2011). There is no adequate level of genetic resistance to BGM in the cultivated genotypes. However, high levels of resistance have been found in the wild *Cicer* species, including *C. judaicum*, *C. bijugum*, *C. echinospermum* and *C. pinnatifidum* (Pande et al. 2006c). Thus, several wide and intraspecific hybridizations have been carried out to transfer the identified disease resistance genes in wild types and land races to commonly adopted and widely grown chickpea cultivars.

Other fungal diseases considered of local importance could also affect chickpea productions. Rust (*Uromyces ciceris arietini*) has been reported to be a problem in central Mexico and Italy (Ragazzi 1982; Díaz-Franco and Pérez-García 1995). The resistance is controlled by a single gene (*Uca1/uca1*) (Madrid et al. 2008) and moderate levels of incomplete and partial resistance are available (Rubiales et al. 2001). Phytophthora root rot (caused by *Phytophthora medicaginis*) affects cool season plantings (Chen et al. 2011). Until now, five genotypes (FLIP 97-132C, FLIP 97-85C, FLIP 98-53C, ILC-5263 and NCS 9905) evaluated under controlled conditions in Pakistan exhibited highly resistant response to the disease (Akram et al. 2008). *C. echinospermum* appears to be the most promising source of resistance in wild species after field and controlled condition evaluations in Australia (Knights et al. 2008).

Parasitic plants as broomrape (*Orobanche crenata* and *Orobanche foetida*) may cause serious losses in chickpea productions in winter sowing under Mediterranean conditions (Rubiales et al. 1999; Roman et al. 2007). Sources of resistance to *O. crenata* in Spain (Rubiales et al. 1999) and to *O. foetida* in Tunisia (Amri personal communication) have been identified. Despite the low broomrape infestation levels observed in chickpea compared to other grain legume species, rapeseed or wild species recently, more aggressive and virulent new *Orobanche* populations are arising (Amri et al. 2009). Field dodder (*Cuscuta* spp.) is another parasite that was reported damaging chickpea production in many regions in the world (Goldwasser et al. 2012a; Chen et al. 2014). In highly infested fields, this parasite can cause up to 100% loss in grain production (Singh et al. 2007). Sources of resistance for *Cuscuta campestris* (field dodder) (ICCV 95333 and Hazera 4) exhibiting high resistance were identified in Israel (Goldwasser et al. 2012b).

In addition, efforts have been made to identify sources of resistance to both pests pod borer (*Helicoverpa armigera* Hübner) and leaf miner (*Liriomyza cicerina* Rondani) in the cultivated and wild species at ICRISAT and ICARDA (Gaur et al. 2007).

5.2 Abiotic Stresses

Terminal drought is globally the most serious abiotic stress to chickpea productivity and the most important factor for instability of yield in major production countries as Asia and Africa, where chickpea is mainly grown as a rainfed crop on residual moisture. Cultivars may escape (early maturity) or tolerate terminal drought increasing the efficiency of water use. Promising accessions (ICC 4958, ICC 1882, ACC 316 and ACC 317) and varieties with a vigorous and deeper root system to improve drought tolerance have been developed (Saxena et al. 1993; Gaur et al. 2008; Cancy and Toker 2009). In addition, transgenic plants have been developed at ICRISAT having either a dehydration responsive element or a gene that increases proline accumulation in the plant (Gaur et al. 2007).

Salt stress imposes a significant limitation of productivity related to the adverse effects on the dry weights of both shoots and roots and also on nodulation and nitrogen fixation (Manchanda and Garg 2008). Limited efforts to identify salinity tolerance within chickpea indicated low genotypic variation and few varieties

with tolerance to moderate levels of salinity (ECe ranging from 4 to 6 dS/m) have been developed. Karnal Chana 1 (CSG 8962) and Genesis 836 (ICCV 96836) were developed in India and Australia, respectively (Maliro et al. 2004). Recently, ICRISAT identified several lines that gave a higher yield than the salinity tolerant cultivar Karnal Chana 1 (Krishnamurthy et al. 2011; Gaur et al. 2012).

Finally, both freezing ($<-1.5^{\circ}\text{C}$) and chilling (between -1.5 and 15°C) are known to affect chickpea at various development stages from germination to maturity (Croser et al. 2003) and should be considered in chickpea breeding for winter sowing in Mediterranean environments. Two chilling tolerant cultivars ('Sonali' and 'Rupali') have been released in Australia (Clarke et al. 2005) and should be included in winter sowing breeding programmes to avoid problems in pod filling in fresh spring. Also, ICARDA and ICRISAT breeding programmes developed cold-tolerant cultivars adapted to winter sowing (Gaur et al. 2007).

5.3 Phenological Characters

Flowering time is influenced by photoperiod and temperature and is a major task to improve crop adaptation. Early flowering associated with early maturity is preferred to escape from terminal drought, high temperature or frost at the end of the season (Gaur et al. 2007). A moderate and positive genetic correlation between days to flowering and seed weight was reported by Hovav et al. (2003), suggesting that it is difficult to breed early-flowering cultivars without compromising seed weight. However, ICRISAT developed two extra large and early-maturing kabuli types from Mexican origin, which suggests that it is possible to breed early varieties with extra large seeds (Gaur et al. 2006). The first landmark variety was ICCV 2, which matures in about 85 days, and it is perhaps the world's earliest maturing variety of kabuli chickpea. Two super-early desi chickpea lines, ICCV 96029 and ICCV 96030, which mature in 75–80 days in southern India were developed by Kumar and Rao (1996). Further advancements have been made in breeding for super-earliness and several short-duration high-yielding varieties of chickpea, both in desi and kabuli types (Gaur et al. 2012).

6 Breeding Methods

Productivity, yield stability in different environment conditions and resistance/tolerance to main damaging diseases are the major goals in chickpea-breeding programmes. These constraints become more pronounced especially with the climate change affecting both the development of the crop and its enemies (development of new pathogens/pests and changing in the aggressiveness and virulence of others). The development of chickpea crop in a sustainable agricultural system is facing several challenges: (i) being more efficient in the development of new varieties resistant/tolerant to main biotic and abiotic stresses and (ii) strict adoption of these new developed varieties by farmers that could result in an improved productivity, reducing yield fluctuations.

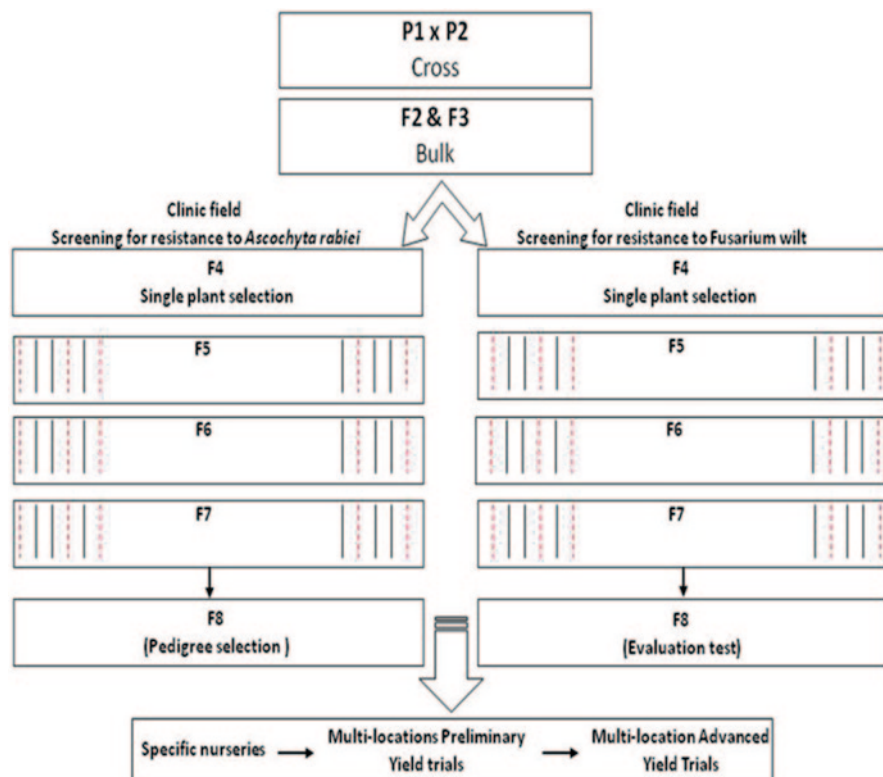


Fig. 3.3 Combined bulk and pedigree method in chickpea-breeding for tolerance/resistance to both *Ascochyta* blight and *Fusarium* wilt diseases (chickpea-breeding programme—Tunisia). Seeds coming from the same selected plant are sown in both blight and wilt clinic fields during the same cropping season. Crosses are mainly performed at ICARDA

Any breeding programme can be achieved through three main steps or components: (i) the genetic variation which is the base of the breeding programme, (ii) strict and rigorous selection within that variation and (iii) evaluation and confirmation of the selected lines (Salimath et al. 2007). Chickpea can be considered a strict self-pollinated crop. Hence, inbreeding to fix genes and develop pure-line cultivars are the main breeding objectives. Mass or pure-line selection from landraces was the simplest method initially employed. Later, crossing programmes and subsequent different modification of pedigree methods and backcrossing programmes for qualitative traits have been developed (Gaur et al. 2012). Most chickpea-breeding programmes have been confined to intraspecific hybridization that includes desi × desi, kabuli × kabuli or desi × kabuli crosses (Gaur et al. 2007). As mentioned in Sect. 3 of this chapter, desi and kabuli types have different genetic backgrounds differing in disease resistance, tolerance to abiotic stresses and quality components. Depending on the objective of the breeding programme, single, three-way or multiple crosses are used. Single crosses have been the most widely adopted. Figure 3.3 shows an example of the methodology followed in the national chickpea-breeding

programme in Tunisia at the National Institute of Agronomy Researches/Regional Field Crop Research Center of Beja (INRAT/CRRGC) while the crosses are mainly performed at ICARDA. Hybridization also allowed the obtention of recombinant inbred lines (RILs) by single-seed descent (SSD) methods (Johnson and Bernard 1962) where plant materials combining interesting traits have been selected (Rubio et al. 2006). Three-way or multiple cross approaches provide additional opportunities to study gene interactions with the aim of combining different traits from different parents in a new cultivar. Hybridization allows the exploitation of transgressive inheritance of some characters such as yield or seed size.

Singh (1987) summarized the effectiveness and specificity of various breeding methods in respect to the desired specific traits and reported that: (i) pedigree method could be used for resistance to biotic stresses; (ii) bulk-pedigree method for drought tolerance and winter hardiness; (iii) modified bulk method for abiotic stresses, seed size, earliness and plant type; (iv) backcross method for inter specific hybridization and (v) limited backcross for desi × kabuli introgression and resistance breeding. Pedigree methods are not frequently used due to the cumbersome data collection and the fact that this approach limits the breeding programme to only a few crosses. The combination of bulk and pedigree methods is widely used among many chickpea-breeding programmes (Gaur et al. 2012). The backcrossing is in general applicable to incorporate one or few oligogenic characters into a well-adapted elite variety. Thus, tolerance to *Ascochyta* blight and double podding were successfully introgressed into CDC Xena, CDC Leader and FLIP98-135C using markers linked to the quantitative trait loci (QTLs) for both traits (Taran et al. 2013).

Introgression from the wild related species into the cultigens has been also employed. Beneficial traits such as cold tolerance and a high degree of resistance to wilt, root rot, and *Botrytis* grey mould have been introgressed from *C. reticulatum* and *C. echinospermum* into cultivated chickpea (Singh et al. 2005; Ramgopal et al. 2012). Similarly, the *C. reticulatum* accession ILWC 119 was included in a crossing programme giving two cyst nematode resistant chickpea germplasm lines ILC 10765 and ILC 10766 (Malhotra et al. 2002).

7 Integration of New Biotechnologies in Breeding Programmes

The integration of genomic technologies in chickpea breeding will greatly improve the efficiency and time required of breeding programmes in the development of better cultivars (Gaur et al. 2012; Castro et al. 2013).

7.1 Genetic Maps

Molecular markers are considered valuable tools for crop improvement due to their usefulness in characterizing and manipulating genetic loci responsible for monogenic and polygenic traits. Markers have made it possible for the development

of genetic maps, the necessary framework for any marker-assisted selection (MAS) programme. Availability of well-saturated genetic linkage maps is a prerequisite for tagging traits with molecular markers, thus enabling their use in MAS and positional cloning of genes of interest.

In chickpea, the first published maps were developed analyzing isozymes in F_2 populations derived from interspecific crosses (Gaur and Slinkard 1990; Kazan et al. 1993). Since then, tremendous advances in DNA marker technology and map development have been achieved, allowing for the identification of markers close to genes or genomic regions with agronomical importance. Even though maps are still incomplete, the chances of finding new polymorphic markers have been considerably increased, essentially due to the development of STMS markers (Hüttel et al. 1999; Winter et al. 1999; Sethy et al. 2003, 2006; Lichtenzweig et al. 2005; Choudhary et al. 2006; Nayak et al. 2010). The use of STMS markers in different populations gave the possibility of exchanging information between maps. These markers have also made it possible to build a consensus chickpea map (Millán et al. 2010) following the nomenclature previously proposed by Winter et al. (2000), which has been considered as the map of reference. Nayak et al. (2010) completed the map published by Winter et al. (2000) adding 175 new markers and provided anchor points for comparing chickpea linkage groups with linkage groups in the model species *M. truncatula*. More recently, the development of next-generation sequencing technologies allowed the obtaining of the first chickpea transcriptome (Hiremath et al. 2011). Large-scale molecular markers were obtained using the transcriptome information and comprehensive genetic maps were developed (Gujaria et al. 2011; Thudi et al. 2011; Hiremath et al. 2012; Jhanwar et al. 2012). High-density genetic maps of gene-based markers represent a powerful resource to enhance genome analysis, thus providing an important opportunity to directly tag genes related to agronomical traits. Due to the low levels of genetic diversity present within the gene pool of cultivated chickpea, the high-density genetic maps have been developed in the interspecific cross ICC 4958 \times PI 489777 (Table 3.1), considered as

Table 3.1 Second generation genetic maps developed in chickpea recombinant inbred lines (RIL) populations

Reference ^a	Newly developed markers	No. of loci	Coverage (cM)	Average inter-markers distance (cM)
Gujaria et al. (2011) ^a	SSR, GMMs, CISR	300	766.56	2.55
Thudi et al. (2011) ^a	BES-SSR, DarT	1291	845.56	0.65
Choudhary et al. (2012) ^a	EST-SSR, ITPs, SNPs	406	1497.7	3.68
Gaur et al. (2012) ^a	SNPs, SSR	368	1808.7	1.7
Hiremath et al. (2012) ^a	CKAMs, TOGs-SNPs	1328	788.6	0.59
Stephens et al. (2014) ^b	SSRs, SNPs	401/417	658.7/752	1.74/2.16

^a RIL derived from *Cicer arietinum* (ICC 4958) \times *C. reticulatum* (PI 489777)

^b RIL derived from Lasseter \times ICC3996/ S95362 \times Howzat

GMMs genic molecular markers, SSR simple sequence repeat, EST expressed sequence tag, SNP single-nucleotide polymorphism, CKAMs chickpea KASPar assay markers, TOGs tentative orthologous genes, ITPs intron targeted primers, BES bacterial artificial chromosome (BAC)-end sequences

the reference mapping population. As far as we know, this population was only phenotyped for wilt (races 4 and 5, Winter et al. 2000). So, the high-resolution map developed in this cross could not be used for tagging the genomic regions associated to other agronomic traits, such as *Ascochyta* blight, which is the most important constraint in chickpea. Recently, the construction of two intraspecific genetic maps has been reported (Stephens et al. 2014) using the RIL populations Lasseter \times ICC 3996 and S95362 \times Howzat, both segregating for blight resistance. In addition to the reference interspecific map, it may be very useful performing a high-resolution mapping in new crosses segregating for more characters valuable for breeding applications.

Moreover, the availability of the draft genome in desi and kabuli chickpea has opened the possibility of anchoring genetic maps and positioning QTL on the physical one (Jain Rajesh et al. 2013; Varshney et al. 2013; Madrid et al. 2014). The identification of markers with complete association with QTLs will boost the development of “perfect” markers in pulses (Kumar et al. 2011). Such markers are extremely useful for guiding the introgression of multiple genes, because they increase selection efficiency and avoid recombination events between markers and QTLs (Hospital 2009).

Major efforts of breeding programmes are concentrated in the development of resistant lines to the main fungi affecting crops (*Ascochyta* blight and *Fusarium* wilt). The majority of authors consider the resistance to blight as a quantitative trait and several QTLs have been identified in the chickpea genetic map (Millan et al. 2013). QTLs for resistance to blight have been located and validated on linkage groups LG4 (QTL_{AR1} and QTL_{AR2}), LG2 (QTL_{AR3}), LG3 (QTL_{AR4}) and LG8 (QTL_{AR5}) of the chickpea map employing different mapping populations (Millan et al. 2013). Another QTL was also detected in LG6 using the cross ICCV 96029 \times CDC Frontier (Anbessa et al. 2009).

Resistance to *Fusarium* wilt in chickpea has been described to be race specific and controlled by major resistance genes, the majority of which are recessive in nature. Resistance genes to races 0, 1, 2, 3, 4 and 5 (*foc-0₂*, *foc-1*, *foc-2*, *foc-3*, *foc-4* and *foc-5*) have been found to form a cluster located on LG2 of the chickpea map (Sharma and Muehlbauer 2007; Cobos et al. 2009; Gowda et al. 2009; Halila et al. 2010). However, one of the two resistance genes for race 0 (*foc-0₁*) was found in LG5 (Cobos et al. 2005) confirming that the resistance is controlled by two independent genes (*foc-0₁* and *foc-0₂*), as Rubio et al. (2003) reported before by classical genetic studies.

Markers associated with other diseases as rust and BGM have been localized. A gene that controls resistance to chickpea rust (*Ucal/ucal*) has been located in LG7 tightly flanked by two STMS markers (Madrid et al. 2008). On the other hand, three genomic areas controlling resistance to BGM have been identified by Anuradha et al. (2011). QTL1, sited in LG6, explained 12.8% of the total phenotypic variation while QTL2 and QTL3 explained 9.5 and 48%, respectively, both of them located in LG8 (names of the groups are referred to chickpea consensus map).

In addition, several important characteristics such as quality components and agronomic traits have also been mapped and flanking markers were identified.

Pigmentation of the flower (pink/white= $P/p=B/b$), brown/yellow seed testa ($T3/t3$), purple/green epicotyl (Gst/gst) and seed coat thickness (Tt/tt) are some of the phenotypic traits with simple inheritance mapped in the chickpea genetic map and located in LG4 (Tekeoglu et al. 2002; Cobos et al. 2005). Erect/prostrate plant growth habit (Hg/hg) is another trait with simple inheritance which has been reported to be located in LG3 (Winter et al. 2000; Cobos et al. 2009). Double pod is a mutation controlled by a single recessive gene designated as s or sfl (Muehlbauer and Singh 1987) linked to TA80 (Rajesh et al. 2002) in LG6 (Cho et al. 2002; Cobos et al. 2005). Regarding yield, different QTLs have been identified in LGs 2, 4, 5 and 7 by different authors (Cobos et al. 2007; Gowda et al. 2011). Early flowering is another trait related to yield improvement. It seems to have a positive effect on yield under the Mediterranean environment (Siddique et al. 2003; Rubio et al. 2004). QTLs controlling this trait have been detected in LGs 1, 2, 3, 4 and 6. The QTLs with high limit of detection (LOD) values in LG3 have special interest for breeding applications as they were validated in different environments and in both intra- and interspecific populations (Cho et al. 2002; Cobos et al. 2009). A recent study published studying drought tolerance identified 45 robust main-effect QTLs (M-QTLs) explaining up to 58.20% phenotypic variation and 973 epistatic QTLs (E-QTLs) explaining up to 92.19% phenotypic variation for several target traits. Nevertheless, there is a *QTL-hotspot* in LG4 explaining about 58.20% phenotypic variation containing seven markers (ICCM0249, NCPGR127, TAA170, NCPGR21, TR11, GA24 and STMS11) that could be further used in MAS (Varshney et al. 2014b).

7.2 Marker-Assisted Breeding

Molecular markers closely linked to a particular agronomic trait facilitate the detection of favourable alleles in breeding programmes. MAS is particularly useful in the case of breeding for disease resistance in order to avoid complex and time-consuming evaluations as well as for pyramiding different resistance genes in the same genotype. However, the efficacy of MAS relies on the saturation of genomic areas of interest with robust, highly polymorphic, easy to interpret and cost-effective markers (Collard and Mackill 2008).

Despite the effort carried out during the past years to saturate genetic linkage maps and identified markers tightly linked to traits of interest in chickpea, the adoption of MAS in chickpea breeding has not been widely employed. Most cultivars of chickpea are the results of conventional plant-breeding programmes, where trait evaluation and phenotypic selection under field or greenhouse conditions are the routine procedure. With the advent of molecular markers and genetic maps, there has been an increased interest in the use of marker technology to facilitate chickpea crop improvement. As previously mentioned, molecular markers have been used for identification and mapping of genes and QTLs for agriculturally important traits in chickpea. However, the extent to which markers have to be employed in chickpea-breeding programmes has not been clearly determined. To date, only few studies

reported the employment of MAS in conventional chickpea-breeding programmes (Taran et al. 2013; Varshney et al. 2014a). In addition to the employment of molecular markers in breeding programmes, they can be used to develop new genomic resources to be used in genomics studies or to introduce new genetic combinations in the programmes. For example, Castro et al. (2010) employed the most associated marker with the *Foc5* gene located in LG2 (TA59) to assist the selection of resistant or susceptible genotypes in order to develop near isogenic lines (NILs).

Regarding Ascochyta blight, the sequence characterized amplified region (SCAR) markers SCY17₅₉₀ and SCAE19₃₃₆, tightly linked to QTL_{AR2}, were successfully employed to tag a source of Ascochyta blight resistance in a collection of chickpea genotypes (Imtiaz et al. 2008). Recently, a new codominant molecular marker (CaETR) was developed by Madrid et al. (2013) based on allelic sequence length polymorphism in an ethylene receptor-like gene located in the QTL_{AR1} (Madrid et al. 2012). It was probed for the usefulness of the markers SCY17₅₉₀ and CaETR to discriminate between resistant and susceptible chickpea genotypes. Those markers have been used in other studies in order to check its efficiency in MAS of blight-resistant genotypes in different breeding programmes (Bouhadida et al. 2013; Castro et al. 2013). These two markers contribute efficiently in the selection of new chickpea varieties with better combinations of alleles to ensure resistance to Ascochyta blight.

Recently, the use of molecular markers in a backcrossing breeding programme (MABC) was reported. Markers were applied to introgress blight resistance (LG4b and LG8) and double podding (LG6) into adapted chickpea cultivars (Taran et al. 2013). In addition, MABC has been used to introgress resistance to Fusarium wilt race 1 and Ascochyta blight in C214, an elite cultivar of chickpea (Varshney et al. 2014a). This approach permits the selection of plants with more than one set of QTL for resistance to blight and double podding without phenotyping.

In spite of these efforts of using molecular markers in breeding programmes, it is imperative to characterize molecular markers useful for MAS targeting the most important agronomic traits, such as Fusarium, Ascochyta, yield, growth habit, etc.

On the other hand, the possibilities for genetic improvement and selection approaches are limited in chickpea when stress tolerance is present in sexually incompatible gene pools. To solve this problem, transgenic chickpeas have been developed. Transgenic chickpeas expressing either the *cry1Ac/b* or the *cry2Aa* gene and the bean-amylase inhibitor gene are resistant to *Helicoverpa* and bruchids, respectively, but these chickpeas have yet to be commercialized. Unfortunately, attempts to generate transgenic chickpeas with increased tolerance to drought and salinity or with increased methionine content have been less successful (Acharjee and Sarmah 2013).

7.3 Functional Genomics

The aim of functional genomics is to discover the biological function of genes and to determine how sets of genes and their expressed products interact in a particular

phenotype. Understanding the functional characteristics and expression of a particular trait may also involve techniques such as expressed sequence tag (EST) library construction, mutant identification, RNA interference (RNAi) experiments and overexpression studies.

Preliminary investigations have been carried out in chickpea to determine important functional genes involved in traits such as abiotic tolerances (Mantri et al. 2007), seed quality (Gremigni et al. 2004) and biotic disease resistances (Jaiswal et al. 2004; Coram and Pang 2005a, b). Until now, transformation studies on chickpea are preliminary (Indurker et al. 2010; Acharjee and Sarmah 2013; Kanakala et al. 2013), and no RNAi essays have been performed. The most detailed functional studies have been made using an enriched library of EST sequences, microarray experiments or Supersage studies (Coram and Pang 2005a, b, 2006; Molina et al. 2008, 2011). Nevertheless, these studies did not establish correlation between the genes identified and the genomics regions genetically mapped related with agronomic traits. With the advent of the complete genome sequence, the identification of genes directly located on these regions will be within reach in an easy way.

One of the most common techniques to perform functional genomic studies is the real-time quantitative polymerase chain reaction (qPCR) technology. It has emerged as the most accurate and sensitive method for gene expression analyses (Derveaux et al. 2010). To perform these studies it is necessary to validate internal control genes first. In chickpea, different combinations of reference genes were given depending on the stress under study (Castro et al. 2012b) enabling an accurate and reliable normalization of qPCR results.

8 Seed Production

In the past years, new successful chickpea varieties have been originated over the world mainly by international or national research institutions or growers associations. The profit margin from chickpea seeds is low and, generally, does not attract private sector investment because chickpea is highly self-pollinated and many farmers use their own seeds stored on farm (Van Gastel et al. 2007). This is the common situation for small farmers in developing countries, where food legumes are very important in family nutrition, but, generally, they do not have access to seeds from improved food legume varieties. In contrast, developed countries such as the USA, Canada or Australia, mainly exporters, require high-quality seeds to be able to provide homogeneous raw material to be processed by the industry. Typically, seed quality parameters in chickpea were focused on seed size, shape and seed coat colour, but nowadays the demand of new varieties suitable for pre cooked or processed chickpea seeds is increasing. Chickpea seed production has been widely reviewed by Van Gastel et al. (2007), who described seed classes following the Organization for Economic Co-operation and Development (OECD) nomenclature. In general, new varieties obtained in chickpeas are pure lines, but still a small amount of heterozygosity could be present in the breeder or foundation seed. Around 500

selected plants from each variety should be harvested and threshed separately to initiate variety seed production. The next step should be sowing seeds from each plant in a single progeny row in order to discard rows with off-type plants.

Careful crop management practices such as sowing in uniform fields should be applied. In addition, requirements for previous cropping in the seed field should specify the crops that should not be grown for a limited time preceding the production of the seed crop. In chickpea, the land selected to produce seeds should be free of any other chickpea variety for at least 2 years for pre-basic and basic seeds. For certified seeds, only 1 year between two crops of different varieties is required (Van Gastel et al. 2007). A minimum isolation distance of 1–2 m between two fields is considered to be enough. However, slightly longer isolation distances are recommended for pre-basic seed and 3 m for basic and certified seeds. It is also suggested to use a relatively high plant population density to improve the competitive ability of chickpea plants to weed (Van Gastel et al. 2007).

Seed storage conditions are other important factors to take into account. Reduced moisture and low temperature increase the longevity of the seed. Storing seeds at less than 13% moisture, however, has adverse effects on viability (Siddique and Krishnamurthy 2014). Seed standards (physical purity, percentage of germination, pest and diseases) have not exactly the same parameters in each country. Harmonizing seed certification procedures to develop a flexible or internationally acceptable seed certification scheme should be desirable for the benefit of the national seed industries (Van Gastel et al. 2007).

Possibly, chickpea seed producers associations will play in the future a major role in enhancing adoption of improved chickpea cultivars in developing countries as occurred in Ethiopia, the largest producer, consumer and exporter of chickpea in Africa. In this country, 90% of the seed demand is being met by the farmers organized as seed growers (Fikre 2014).

9 Conclusions and Future Prospects

Chickpea crop has a promising future. It is already a basic food in many Asian countries and it is recognized as a source of biologically active compounds (Roy et al. 2010). It is also a crop with low inputs adapted to low water requirements. However, chickpea belongs to the category of “low value seed crop” because it is highly self-pollinated; so in most cultivated areas, farmers continue to grow old varieties and landraces using their own sowing seeds (Gaur et al. 2010). Research on chickpea crop has been done with successful results in international and national institutes but it is necessary to solve the transference of knowledge to private sector and to solve commercialization of the new varieties.

In spite of the progress made in the last years in the developing of genetic maps and the identification/location of different genes/QTLs related with the main agronomic traits affecting chickpea, the molecular basis of these traits remains unknown. Isolation and validation of genes underlying the genes/QTL for the traits of interest is an essential step to determine gene function. Development of a genome-wide

physical map or local physical map around the gene/QTL region and then sequencing those are the next steps in this direction (Gaur et al. 2012). The availability of the reference genome for desi and kabuli types is facilitating this approach (Madrid et al. 2014) and will allow the development of diagnostic markers enhancing the adoption of molecular breeding for increasing chickpea productivity.

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